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Environmental Effects of Dredging Technical Notes

A Chronic Sublethal Sediment Bioassay with the Marine Polychaete *Nereis (Neanthes) arenaceodentata*

Purpose

This note provides a general overview of a new 28-day chronic sublethal sediment bioassay designed for the regulatory evaluation of dredged material. The bioassay uses survival and growth rate endpoints with the polychaete *Nereis (Neanthes) arenaceodentata*. The primary technical reference for this new bioassay is Dillon, Moore, and Reish (in press), upon which this overview is based.

Background

Sediment bioassays are used to assess the aggregate toxicity of sediment-associated anthropogenic chemicals. Historically, these bioassays have measured survival of highly sensitive species following acute exposures (10 days). A new generation of sediment bioassays is being developed in which the subtle, sublethal response of test species is measured following chronic sediment exposures (Dillon 1993).

This sediment bioassay was developed for the regulatory evaluation of dredged material under section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law (PL) 92-532) and section 404(b)(1) of the Federal Water Pollution Control Act of 1972 (PL 92-500), as amended. The bioassay, which utilizes both survivorship and growth endpoints, was designed specifically to assess the toxicity of bedded sediments. Research and test development were targeted for eventual use by the commercial bioassay contracting community. Thus, great emphasis was placed on logistical feasibility, practicality, and low capital start-up and operating costs.

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To maximize regulatory utility, interpretive guidance explaining the biological importance of test results was also developed. Although targeted for dredged material toxicity testing, this bioassay can be used in other assessments of sediment quality, including bioaccumulation potential, suspended sediment toxicity, and hazard and risk assessments. A more detailed description of this bioassay is given in Dillon, Moore, and Reish (in press).

Additional Information

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Test Organism

Natural History

The test organism for this sediment bioassay is the nereid polychaete, *Nereis (Neanthes) arenaceodentata*, hereafter referred to as *Neanthes arenaceodentata*, the name most familiar to toxicologists. *Neanthes arenaceodentata* is widely distributed in shallow marine and estuarine benthic habitats of Europe, North America, and throughout the Pacific (Day 1973, Pettibone 1963, Reish 1957, Taylor 1984, Whitlatch 1977). *Neanthes arenaceodentata* constructs one or more mucoid tubes in the upper 2 to 3 cm of sediment. This deposit-feeder ingests particles up to 70 µm in diameter with a preference for those around 12 µm (Whitlatch 1980).

Life Cycle

The life cycle of *N. arenaceodentata* is well documented (Figure 1) (Reish 1957, Pesch and Hoffman 1983). As worms approach sexual maturity, males and females establish pairs and occupy a common tube. Eggs are deposited by the female within the tube; the male presumably fertilizes the eggs at this time. The spent female soon exits the tube and dies within 1 to 2 days or is eaten by the male. The male remains in the tube to incubate and guard the developing embryos. Development is direct and occurs entirely within the parental tube. Emergent juveniles (EJs) exit the parental tube about 3 weeks after egg deposition. They establish tubes of their own and begin to feed. Juvenile worms grow, and eggs become visible in the coelom of females at about 6 weeks postemergence. The eggs continue to grow in the coelom, and deposition occurs 9 to 13 weeks postemergence to complete the life cycle. The entire life cycle can be completed in the laboratory in 12 to 17 weeks at 20 to 22 °C.

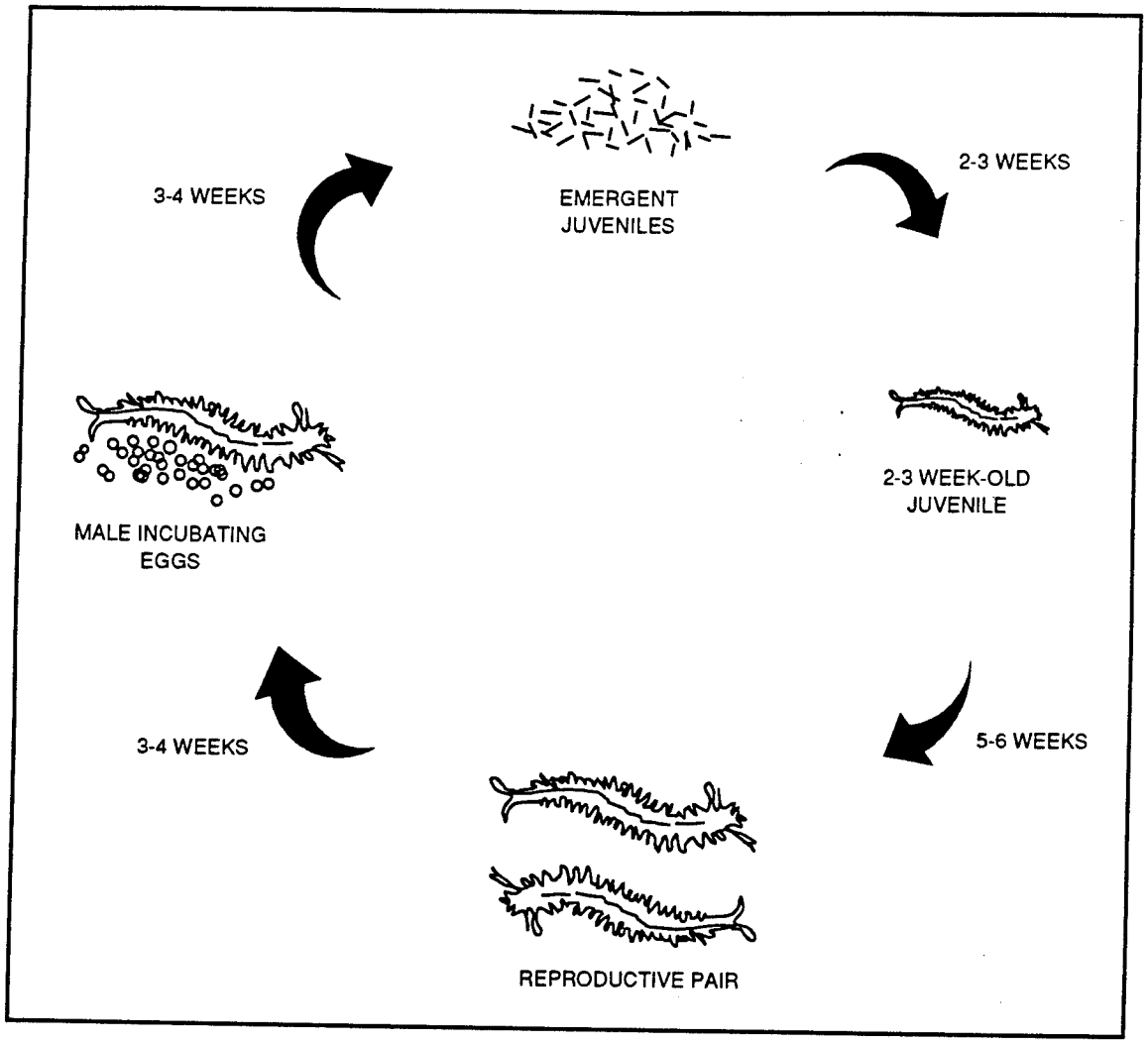

Figure 1. Life cycle of *Neanthes arenaceodentata*

Laboratory Culture Methods

Laboratory cultures of *N. arenaceodentata* were begun at Waterways Experiment Station (WES) in March 1988, from animals provided by Dr. Don Reish of California State University, Long Beach. Worm cultures have been maintained continuously at WES since that time. Worms are maintained at 20 °C in 30 parts per thousand (ppt) artificial seawater made up with reverse osmosis water (ROW). The photoperiod is 12 hr light. EJs are raised to adulthood in 38-L all-glass aquaria (100 EJs/aquarium) containing 30-L aerated seawater and a 2- to 3-cm layer of fine-grain, uncontaminated marine sediment collected near Sequim, WA. Twice weekly, finely ground (≤0.50 mm) Tetramarin (100 mg) and alfalfa (50 mg) are added to each aquarium via a seawater slurry.

After 10 weeks, worms are paired using the intrasexual fighting response and the presence/absence of eggs in the coelom (Reish 1974). Pairs are placed in 600-ml beakers with 500 ml of seawater. Each pair is initially fed a slurry containing 4 mg Tetramarin and 4 mg alfalfa. Beakers are monitored daily for

the presence of eggs and EJs. When discovered, EJs are pooled from different broods and returned to the 38-L aquaria to complete the culture cycle.

Toxicology

N. arenaceodentata is recommended for dredged material toxicity testing by the two Federal agencies having regulatory responsibility—the U.S. Army Corps of Engineers (USACE) and the U.S. Environmental Protection Agency (USEPA) (USEPA/USACE 1991, 1993). It has been used in numerous scientific studies designed to evaluate the chronic sublethal effects of contaminated sediment (Chapman and others 1992; Johns, Gutjahr-Gobell, and Schauer 1985; Johns, Pastorok, and Ginn 1991; Pastorok and Becker 1990; Pesch, Mueller, and Pesch 1987; Tay and others 1992). A considerable amount of information has also been reported regarding contaminant-specific toxicity for this species (Moore, Dillon, and Suedel 1991; Reish 1980, 1985).

Statistical Design

The null hypothesis for this sediment bioassay is that there are no statistically significant ($\alpha = 0.05$) differences between the project or test sediments and the reference sediment. Characteristics and selection of an appropriate reference sediment are discussed in USEPA/USACE (1991, 1993). There are five replicates per treatment and five animals per replicate.

Test Protocol

This section describes the protocol for conducting the 28-day chronic sublethal sediment bioassay with *N. arenaceodentata*. General guidance for conducting dredged material toxicity tests can be found in USEPA/USACE (1991, 1993). Supporting information can also be found in Standard Guides produced by the Sediment Toxicology Subcommittee E47.03 of the American Society of Testing and Materials (ASTM 1991a,b).

Sediment Handling and Exposure Vessel Preparation

Sediments are stored cold (4 °C) in sealed containers with a minimum of overlying water. One to 2 days before initiating the bioassay, sediments are removed from cold storage and press-sieved (2-mm screen) without the addition of seawater. After sieving, sediments are thoroughly homogenized. Replicate subsamples are removed from the homogenized sediment for grain size analysis and interstitial salinity, pH, and ammonia determinations. Enough sediment is added to each 1-L beaker to create a 2- to 3-cm layer. After sediment has been added to prelabeled beakers, 30-ppt seawater is slowly added to the 800-ml mark in a manner that minimizes sediment resuspension. Beakers are placed in a temperature- and photoperiod-controlled environment (20 °C and 12 hr light, respectively). Trickle-flow aeration is provided via glass pipette (suspended 2 to 3 cm above the sediment) after any suspended sediment has

settled. Beakers are covered with watch glasses to minimize evaporation and keep out any dust.

Test Initiation

Initial Water Quality. Overlying water should be carefully renewed prior to test initiation. Following this renewal, but prior to the addition of worms, overlying water quality should be determined in each beaker. At a minimum, temperature, salinity, dissolved oxygen, pH, and ammonia should be determined.

Test Organisms. Juvenile worms (2 to 3 weeks old) used to initiate this sediment bioassay are selected from a pool containing about twice the number of animals needed. Selected worms are randomly placed in prelabeled 100-ml holding beakers (five worms per beaker) containing seawater. The number of holding beakers prepared should be sufficient for the sediment bioassay (five beakers per treatment), initial dry weights (five beakers), and the reference toxicant test (30 beakers).

The bioassay is initiated when worms are introduced into the 1-L exposure beakers containing sediment. Worms are added one at a time to verify the initial census and to visually examine the condition of each worm to ensure the inclusion of representative, undamaged worms.

Feeding. Once the bioassay is initiated, each beaker is provided a seawater slurry containing finely ground (≤0.50-mm) well-hydrated Tetramarin (5 mg) and alfalfa (2.5 mg).

Initial Dry Weights. Initial dry weights are determined on a subsample of 25 worms from the pool of animals used to initiate the bioassay. These worms are placed in five beakers during the selection of test animals (see above). Procedures for dry weight determinations are described below (see **Test Termination** section).

Reference Toxicant Test. A seawater-only 96-hr reference toxicant test with cadmium chloride is conducted at the same time the sediment bioassay is initiated. WES researchers currently use six exposure concentrations (0, 3, 6, 12, 24, and 48 mg Cd/L), five replicate beakers per concentration, and five worms per beaker. Experimental conditions are the same as in sediment bioassays. Worms for the reference toxicant test are drawn from the same pool of animals used to initiate the sediment bioassay. Worms are not fed during the test. After 96 hr, the number of survivors in each beaker is recorded. Water quality is determined in each beaker when the test is initiated and at termination. A 30-ml sample is collected from each beaker initially and at test termination to analytically confirm nominal cadmium concentrations.

Test Maintenance

Each beaker is visually checked every weekday. Abnormal and/or unanticipated events and observations are recorded in the lab notebook. Weekly

seawater renewals are sufficient to maintain good water quality. At each renewal, approximately 80 percent of the overlying seawater is removed and refilled to the 800-ml mark. Water quality (dissolved oxygen, salinity, pH, ammonia) should be monitored in each beaker prior to each renewal and at test termination. Temperature should be monitored daily. Worms are fed the Tetramarin-alfalfa slurry (described above) twice weekly, after every renewal, and 3 to 4 days later.

Test Termination

The test is terminated after 28 days of sediment exposure. A final visual check of each beaker is made, and terminal water quality is assessed. All surviving worms are removed from each beaker by sieving the sediment through a series of stacked screens (2.0-, 1.0-, and 0.5-mm mesh size). The number of surviving worms per replicate is recorded. All surviving worms from a replicate are briefly rinsed in ROW to remove saltwater and any adhering sediment, pooled, and placed on a tared aluminum weighing pan. Tissue samples are oven-dried at 60 °C to a constant weight (about 24 hr), brought to room temperature under desiccation, and reweighed. Estimated individual worm weights are calculated by dividing the total dry weight biomass in a replicate by the number of survivors. Growth rate (milligrams per day) over the period of the bioassay is calculated by subtracting estimated initial weight from estimated individual final weight and dividing by the exposure period (28 days).

Data Analysis

Data Validation

Data validation procedures generally assume that data are valid until they deviate from some performance criteria. Significant deviations in performance criteria can be grounds for rejecting data unless a good explanation can be provided. Standard data validation procedures for sediment bioassays have not been formalized. Performance criteria for individual sediment bioassays typically evolve over time in an ad hoc fashion rather than by any rigorous numerical analysis. Based on observations at WES and in other laboratories, it is recommended that data validation be carried out using the following assessments.

Performance Criteria for the Negative Control Treatment. The negative control for most sediment bioassays is beakers containing sediment in which the animals were either cultured or field collected. For this bioassay with *N. arenaceodentata,* the recommended performance criteria are ≥80 percent survival in any one replicate and ≥90 percent mean survival for all replicates containing Sequim Bay sediment. Failure to meet these criteria is grounds for considering the test results invalid.

Performance Criteria for the Positive Control. For this bioassay, the survival of *N. arenaceodentata* in the reference toxicant test with cadmium chloride is

the positive control. Tests conducted thus far indicate that 96-hr LC_{50}s range between 10 and 15 mg/L. These data will eventually be used to construct a Shewart Control Chart, which will be used to identify statistically "out of control" data and, thus, potentially invalid sediment bioassays, when sufficient tests have been conducted. Extant guidance (Environment Canada 1990, Shainin and Shainin 1988) suggests that 15 to 25 tests are required to construct a control chart.

Water Quality Data. Mean water quality should meet the following performance criteria: temperature, 20 °C ± 2 °C; salinity, 30 ppt ± 3 ppt; dissolved oxygen, ≥6.0 mg/L; pH, 8.0 ± 1.0; and total ammonia, ≤1.0 mg/L.

Anomalous Events or Deviations from Good Laboratory Practice. Anomalous events or deviations from good laboratory practice can also be grounds for rejecting data. However, the impact of those events, if sufficient in magnitude or duration, should be reflected in deviations of the above performance criteria.

Statistical Analysis

One-way analysis of variance is used to test the null hypothesis that response in the reference sediment is not statistically different from that in the project sediments. This analysis is conducted for both test endpoints: survival and estimated individual growth rate. Homogeneity of variances is evaluated with either Bartlett's test or Levene's test using appropriate transformations as needed. Normality is evaluated by plotting residuals. Mean separation may be performed via Tukey's HSD test, Dunnett's, or another appropriate parametric procedure. All differences are assumed statistically significant at $P < 0.05$.

Interpreting Bioassay Test Results

A tiered hierarchy for interpreting bioassay test results is recommended, as outlined below.

Tier I: Are the test results valid?

Methods to validate data were discussed above. If the data cannot be validated, and if no reasonable explanation can be provided, test results may be considered invalid. Further analysis would be unwarranted. If the data are acceptable, proceed to Tier II.

Tier II: Are the results statistically significant?

Statistical methods are recommended above. If response in the reference sediment is statistically indistinguishable from that in the project sediments, further data interpretation is unwarranted. If results are significantly different, go to Tier III.

Tier III: Are the results biologically important?

A statistically significant result may or may not be important biologically. For example, if a project sediment causes a statistically significant 5-percent decrease in survival, is that level of response truly detrimental to the organism? Would a 10-percent decrease be twice as "bad" or only incrementally injurious? Deciphering the biological importance of sublethal endpoints such as growth is even more problematic.

For this bioassay, the technical basis for interpreting test results relies on the relationship between growth and reproductive success. Growth and reproduction are energy antagonists. That is, they represent competing demands on a usually limited energy source. As a result, diminished growth will likely lead to adverse effects on reproduction. Establishing the quantitative nature of this relationship provides the technical basis for interpreting the growth endpoint. Moore and Dillon (1993) examined this relationship quantitatively and observed no significant effects on either survival or reproduction when somatic growth rates were ≥0.65 mg (wet)/day. Growth rates ≤0.45 mg/day resulted in significant reductions in reproduction. Very low growth rates (0.05 mg/day) were associated with a nearly complete cessation of reproduction and very poor survival (5 to 11 percent).

Ultimately, the biological importance of ecotoxicology studies should be interpreted in terms of a meaningful population-level response (Barnthouse and others 1986, Bridges and Dillon 1993, Suter 1990). If a contaminant-induced perturbation represents an important environmental hazard, there is a risk that a local population may decline or even become extinct. This risk can be projected quantitatively using demographic population models. These models represent a tool for integrating life history observations (that is, survival, growth, and reproduction) into deterministic and risk-based estimates of population viability (Bridges and Dillon 1993). WES is currently developing a risk-based demographic model for *N. arenaceodentata*.

Quality Assurance (QA)/Quality Control (QC)

QA/QC represents the administrative and technical steps taken to ensure that reliable data are produced with specified precision and accuracy. Specific QA/QC measures associated with this chronic sublethal sediment bioassay with *N. arenaceodentata* were discussed above. Moore and others (1994) provide general QA/QC guidance for conducting dredged material bioassays.

Test "Ruggedness"

ASTM (1992) defines "ruggedness" as the "insensitivity of a test method to departures from specified test or environmental conditions." For sediment bioassays, "ruggedness" is evaluated from two perspectives: sensitivity to the physicochemical properties of sediments and deviations in normal test conditions

and protocols. Examples of the former include the effects of grain size, interstitial ammonia, presence of indigenous fauna, and organic carbon. These factors are known to bias results of acute lethality sediment bioassays (for example as discussed in DeWitt, Ditsworth, and Swartz 1988), and their potential influence will no doubt increase as test duration increases and more sensitive endpoints are examined. The effects of sediment properties and deviations from normal conditions on survival and growth in *N. arenaceodentata* have been examined (Dillon, Moore, and Gibson 1993) and are summarized below.

Intraspecific Densities

Survival was high (81 to 100 percent) after 6 weeks in 600-ml beakers containing sediment and up to 12 juvenile worms. Growth after 6 weeks was unaffected at densities ≤4 worms/beaker but significantly depressed at densities ≥8 worms/beaker. In the absence of sediment, the adverse effects of intraspecific interactions were magnified.

Grain Size

Juvenile worms can tolerate a wide range of grain sizes. Survival was high (89 to 100 percent) and unaffected after 6 weeks in grain sizes ranging from 5 to 100 percent sand. Likewise, there was no significant effect on growth. However, there was a consistent trend of reduced worm weight with increasing grain size. This may indicate a possible grain size effect with longer exposures (>6 weeks).

Salinity

Neanthes arenaceodentata is cultured in 30-ppt seawater at WES. Test sediments may come from areas where the salinity is lower. Survival and growth of *N. arenaceodentata* after 6 weeks was unaffected following acute transfers from 30-ppt seawater to salinities ≥20 ppt. However, no juvenile worms survived acute transfers to ≤15 ppt. The effects of gradual acclimation have not been examined.

Ammonia Toxicity

Juvenile worms exhibited a sharp threshold response to chronic ammonia concentrations, similar to that observed for salinity. Survival and growth were unaffected following a 6-week exposure to total ammonia concentrations ≤10 mg/L. Survival was 0 percent at ≥40 mg/L. At the intermediate test concentration (20 mg/L), both survival and growth were slightly but not significantly diminished.

Hydrogen Sulfide Toxicity

Survival of juvenile worms in short-term (96-hr) exposures was 100 percent at sulfide concentrations ≤5.0 mg/L. At 10.0 and 20.0 mg/L, survival was 44 and 0 percent, respectively.

Resistance to Hypoxia

Survival of juvenile worms in short-term (96-hr) exposures was 100 percent at oxygen concentrations ≥1.5 mg/L. At 1.0 and 0.5 mg/L, survival was 68 and 0 percent, respectively.

Future Activities

Although a test protocol can be recommended at this time, additional test development is required. This work falls into the following categories:

- Conduct bioassays on a wide range of dredged material.
- Continue to evaluate test "ruggedness."
- Evaluate interlaboratory variation.
- Compare with other dredged material bioassays.
- Develop a risk-based demographic population model.

Summary of Bioassay Test Protocol

A protocol for conducting a 28-day sediment bioassay with the marine polychaete *Neanthes arenaceodentata* is described. Primary target application is the regulatory evaluation of dredged material. Bioaccumulation potential may also be evaluated under certain conditions. Test endpoints are survival and estimated individual growth rate. The bioassay is conducted at 20 °C under a 12-hr photoperiod in 1-L glass beakers containing aerated seawater (30 ppt) and a 2- to 3-cm layer of bedded sediment. The test is initiated by randomly adding juvenile worms (2 to 3 weeks old) to beakers (five worms per beaker and five beakers per treatment). Worms are quantitatively fed, twice weekly, a seawater slurry containing finely ground Tetramarin and alfalfa. Seawater is renewed weekly (ca. 80 percent volume replacement). Water quality is monitored at least weekly prior to each renewal and at termination. After 28 days, worms are removed via sieving, and the number of survivors in each beaker is recorded. Survivors from each treatment are pooled, placed on a tared weighing pan, dried to a constant weight (24 hr at 60 °C), and weighed to the nearest 0.01 mg. Estimated individual worm weight is determined for each replicate by dividing total dry weight biomass by the number of survivors. Growth is expressed as a rate (milligrams dry weight per day) for each replicate by subtracting initial dry weight from final values and dividing by the exposure period (28 days). Quality control performance criteria for positive and negative controls are reported. Interpretive guidance for this bioassay is based

on the relationship between growth and subsequent reproductive success. Although designed for the regulatory evaluation of dredged material, this bioassay can be applied to other assessments of sediment quality.

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